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## IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland

The discovery of NOD2/CARD15 as the first susceptibility gene in Crohn's disease has contributed significantly to a fundamental change in the direction of basic research in inflammatory bowel disease (IBD), triggering renewed interest in the integrity of the innate immune response in IBD and appropriate orchestration of a subsequent adaptive immune response.<sup>1,2</sup> More widely, in all complex diseases this finding in 2001 provided a much welcomed and needed proof of principle for non-parametric linkage analysis.

Another study with major implications for the pathogenesis of Crohn's disease as well as for investigation of all complex disorders has recently been published.<sup>3</sup> The North American consortium performed an association study testing 308 332 markers spanning the entire genome in 567 patients with ileal Crohn's disease and 571 controls of non-Jewish European ancestry. Of the three markers reported to retain significance after stringent Bonferroni correction, two were located in the NOD2/CARD15 gene. The third marker (rs11209026) was a non-synonymous variant in the interleukin-23 receptor (IL23R) gene on chromosome 1p31. Replication was obtained in the index paper in a Jewish ancestry case-control analysis of patients with Crohn's disease by transmission disequilibrium testing in 883 families with offspring affected by IBD and in a combined case-control analysis of these three cohorts (IBD,  $p = 6.62E-19$ ).

IL-23 is a pivotal cytokine in the differentiation of T helper cells, especially their differentiation into Th17 T cells.<sup>4</sup> Although the Th17 T cell subset has been shown to mediate chronic and autoimmune inflammatory conditions in animal models, clear evidence exists for the central role of IL-23 in the development of intestinal disease.<sup>5–7</sup>

Both Crohn's disease and ulcerative colitis commonly first present during childhood and adolescence and are associated with high disease-related and treatment-related morbidity in these young patients.<sup>8</sup> Disease incidence is high in our population, and in others in Northern Europe in whom the NOD2/CARD15 contribution is small. These considerations, together with well established epidemiological

**Table 1** Demographic data and inflammatory bowel disease (IBD) phenotype in patients diagnosed with IBD at <17 years of age based on Montreal guidelines for classification of Crohn's disease/ulcerative colitis<sup>9</sup>

N	358
M/F	205/153
Median age at diagnosis (Q1–Q3)	11.1 years (8.6–12.9)
CD/UC/IBD type	239/88/31
unclassified	
Caucasian	97%
Montreal CD location	L1±L4: 13; L2±L4: 81; L3±L4: 131; L4: 3
Montreal CD behaviour	B1(±p): 214; B2(±p): 9; B3(±p): 9; Bx(p): 27
Montreal UC extent	E3: 63; E2: 17; E1: 4

CD, Crohn's disease; UC, ulcerative colitis. Crohn's disease: L1, ileal; L2, colonic; L3, ileocolonic; L4, upper gastrointestinal tract; B1, not stricturing, not penetrating; B2, stricturing; B3, penetrating; p, penetrating perianal. Ulcerative colitis: E3, extensive colitis; E2, left-sided colitis; E1, proctitis only.

data suggesting that early-onset disease has a strong genetic basis, provide a clear scientific rationale for performing molecular studies in this group.

Our aim was to assess the contribution of the Arg381Gln variant (rs11209026) of IL23R in determining susceptibility and phenotype in childhood onset IBD in Scotland. We also sought to investigate the interaction between carriage of any of the three common NOD2/CARD15 variants and carriage of this IL23R variant in determining susceptibility to Crohn's disease.

A total of 1294 subjects comprising 358 with IBD aged <17 years at diagnosis (table 1), 594 parents and 342 controls were genotyped for rs11209026 G/A using TaqMan (7900HT sequence detection system; Applied Biosystems, Foster City, California, USA). Allelic and genotype frequency comparisons between cases and controls using  $\chi^2$  and transmission disequilibrium testing were applied to assess the association of IL23R rs11209026 with IBD. The three common NOD2/CARD15 variants were genotyped as previously described.<sup>10</sup>

In cases and controls, rs11209026 was in Hardy-Weinberg equilibrium. The allelic frequency of rs11209026\*G differed significantly between IBD/Crohn's disease cases and controls (2.9%/3.0% vs 5.5%,  $p = 0.01$ , OR 0.51 (95% CI 0.30 to 0.88) and  $p = 0.04$ , OR 0.53 (95% CI 0.28 to 0.98); table 2).

The GG genotype was associated with an increased risk of IBD/Crohn's disease ( $p = 0.01$ , OR 2.01 (95% CI 1.15 to 3.49) and  $p = 0.03$ , OR 1.96 (95% CI 1.03 to 3.70)). Analysis by transmission disequilibrium testing showed significant overtransmission of the G allele for IBD ( $p = 0.004$ ) and Crohn's disease ( $p = 0.04$ ), with a trend towards significance in ulcerative colitis hindered by a small number of informative families with ulcerative colitis (table 3).

In Crohn's disease there was no difference ( $p = 0.94$ ) in allelic frequency between NOD2/CARD15 wild type and NOD2/CARD15 variant-carrying patients. However, owing to the small numbers of cases and controls carrying this IL23R variant, our study was not adequately powered to formally assess epistasis with NOD2/CARD15. Genotype-phenotype analysis in Crohn's disease and ulcerative colitis based on the Montreal classification did not demonstrate any significant effect of IL23R rs11209026; specifically, we were not able to show a protective effect against ileal Crohn's disease ( $p = 0.21$ ).<sup>9</sup>

The successful identification of IL23R as a novel IBD susceptibility gene has provided proof of principle for the applicability of genome-wide association studies in the genetics of complex diseases. We show for the first time that IL23R variation influences susceptibility to IBD and Crohn's disease, but not phenotype, in an exclusively paediatric IBD cohort. The data complement the results of the initial North American study and the replication studies currently underway in the UK adult population.<sup>10</sup> However, the contribution of this IL23R allele to IBD is not sufficiently strong to explain the high incidence of childhood IBD in our or other populations with a low NOD2/CARD15 contribution. Other determinants are likely to be involved in Northern Europe.<sup>11,12</sup> Genome-wide association scanning has already provided other candidates requiring rigorous analyses.<sup>13,14</sup>

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**Table 2** Interleukin-23 receptor (IL23R) rs11209026G/A (Arg381Gln) genotype and allelic frequencies in controls and patients with inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) diagnosed <17 years of age

Arg381Gln IL23R	Control	IBD	p Value	CD	p Value	UC	p Value
HWE	0.27	0.56		0.63		0.73	
GG	304/342 (88.9%)	337/358 (94.1%)	0.01	219/233 (94.0%)	0.03	80/86 (93.0%)	0.25
GA	38/342 (11.1%)	21/358 (5.9%)	0.01	14/233 (6.0%)	0.03	6/86 (7.0%)	0.25
AA	0	0		0		0	
Allele A	38/684 (5.5%)	21/716 (2.9%)	0.01	14/466 (3.0%)	0.04	6/172 (3.5%)	0.27

Hardy-Weinberg equilibrium (HWE) p values are given.

**Table 3** Interleukin-23 receptor (IL23R) rs11209026G/A (Arg381Gln) transmission disequilibrium testing in trios with childhood onset IBD

Arg381Gln IL23R	Informative families (n)	Transmissions observed	Transmissions expected	p Value
IBD	34	59	50.5	0.004
CD	23	39	34	0.04
UC	9	16	13.5	0.09

Assessed using FBAT software Version 1.7.3 available from <http://www.biostat.harvard.edu/~fbat/fbat.htm>.

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis.

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#### J Van Limbergen

Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, and Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

#### R K Russell

Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

#### E R Nimmo, H E Drummond, L Smith, G Davies

Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, Edinburgh, UK

#### N H Anderson

Public Health Sciences, University of Edinburgh, Edinburgh, UK

#### P M Gillett

Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

#### P McGrogan, K Hassan

Department of Paediatric Gastroenterology, Yorkhill Hospital, Glasgow, UK

#### L Weaver

Department of Child Health, University of Glasgow, Glasgow, UK

#### W M Bisset, G Mahdi

Department of Paediatric Gastroenterology, Royal Aberdeen Children's Hospital, Aberdeen, UK

#### D C Wilson

Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, and Child Life and Health, University of Edinburgh, Edinburgh, UK

#### J Satsangi

Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, Edinburgh, UK

Correspondence to: Dr Johan Van Limbergen, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK; [johanvanlimbergen@hotmail.com](mailto:johanvanlimbergen@hotmail.com)

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## JAK V617F missense mutation is absent in pancreatic cancer

Aberrant constitutive activation of STAT3 (signal transducer and activator of transcription) leads to cellular transformation and aids tumorigenesis in pancreatic cancer and its inhibition can lead to growth arrest.<sup>1-4</sup> The exact mechanism of this constitutive activation

has not been elucidated, although downstream mediators may include ERK and p21 signalling.<sup>1-4</sup> Blocking JAK2 (Janus Kinase), a known upstream activator of STAT3, by AG490, a tryphostin inhibitor, reduced phosphorylation of STAT3 and produced comparable in vitro effects, suggesting a role for JAK2 in pancreatic carcinogenesis.<sup>3</sup>

JAK2 V617F missense mutation has recently been shown by several independent groups to play an important role in myeloproliferative disorders<sup>5</sup> as well as prothrombotic states such as Budd-Chiari syndrome (which may be due to latent myeloproliferative disorders).<sup>5,6</sup> We postulated that JAK2 V617F mutation may play a role in pancreatic cancer, in which the prothrombotic state is well recognised.

We analysed genomic DNA (all from unstained slides of representative surgical specimens) from 26 patients undergoing surgery for various pancreatic diseases (table 1) along with genomic DNA from 10 cell lines (Panc1, Paca3, MiaPaCa2, Capan1 and 2, Suit2, AsPc1, 818A, HpaF2, H766T) for JAK2 V617F mutation using phenol-chloroform extraction with ethanol precipitation, using appropriate positive and negative controls as previously described.<sup>6</sup> Briefly, all cells (>70% tumour tissue in cancer specimens, no microdissection) were scraped from unstained slides into high salt buffer and samples were made up to 200 µl with proteinase K, RNase A and sodium dodecyl sulfate, incubated at 37°C overnight before phenol extraction and ethanol precipitation. Genomic DNA from cell lines was extracted from 70% confluent million cells using Trizol (Invitrogen Ltd, Paisley, UK) reagent digestion followed by ethanol precipitation. A highly sensitive allele-specific PCR was used to detect the G to T mutation in exon 14 of JAK2 (corresponding to amino acid position 617) as previously described.<sup>6</sup> Briefly, a three primer PCR was performed which, in the absence of a mutation, produces an internal amplification control band. Where a mutation is present, an additional band is also amplified and is specific to the mutant sequence. The sensitivity of the assay is 1 cell in 100, determined by mixing positive control genomic DNA (extracted from HEL cells) with non-mutated genomic DNA. We have previously validated this methodology by conventional Sanger sequencing and pyrosequencing.<sup>6</sup> No such mutations were detected at the JAK2 617 codon for pancreatic cancer or cell samples.

We propose that STAT3 activation, as previously described in pancreatic cancer,<sup>1-4</sup> is by mechanisms other than constitutive activation of JAK2 by missense mutation at the V617F site. Similarly, while JAK2 mutation is absent in acute myeloid leukaemia, STAT3 activation is common and is being explored.<sup>7</sup> In glioblastoma, for example, it has been suggested that STAT3 is activated by exogenous interleukin (IL)-4.<sup>8</sup> IL-4 interacts with IL-13Rα2 receptor and, although IL-13Rα2 does not bind with STAT3 directly, it blocks STAT6 activation and excludes this downstream event. Thus, IL-4 can have an anti-apoptotic effect in glioblastoma cells via STAT3 signalling. Conversely, deletion of the SOCS3 (suppressor of cytokine signalling 3) gene in liver cancer<sup>9</sup> and silencing of the SHP1 gene (by methylation) in lymphoma<sup>10</sup> can activate STAT3 signalling. It has recently been suggested that there may be novel mutations in exon 12 which may account for a minority of patients with polycythemia vera and idiopathic erythrocytosis, who are